

Patent claims

1. Nucleic acid molecule comprising a nucleic acid sequence which codes for a haemocyanin, a haemocyanin domain or a functional fragment thereof with the immunological properties of at least one domain of a haemocyanin, and comprising at least one intron sequence, the nucleic acid sequence being selected from:

- (a) nucleic acid sequences which are selected from the group consisting of the DNA sequences shown below or the corresponding RNA sequences or which contain these:

SEQ ID NO:1 (HtH1 domain a + signal peptide),

SEQ ID NO:2 (HtH1 domain b),

SEQ ID NO:3 (HtH1 domain c),

SEQ ID NO:4 (HtH1 domain d),

SEQ ID NO:5 (HtH1 domain e),

SEQ ID NO:6 (HtH1 domain f),

SEQ ID NO:7 (HtH1 domain g),

SEQ ID NO: 8 (HtH1 domain h),

SEQ ID NO:9 (partial HtH2 domain b),

SEQ ID NO:10 (HtH2 domain c),

SEQ ID NO:11 (HtH2 domain d),

SEQ ID NO:12 (HtH2 domain e),

SEQ ID NO:13 (HtH2 domain f),

SEQ ID NO:14 (HtH2 domain g),

SEQ ID NO:15 (HtH2 domain h),

SEQ ID NO:16 (partial KLH1 domain b),

SEQ ID NO:17 (KLH1 domain c),

SEQ ID NO:18 (KLH1 domain d),

SEQ ID NO:19 (partial KLH1 domain e),

SEQ ID NO:20 (KLH2 domain b),

SEQ ID NO:21 (KLH2 domain c),

SEQ ID NO:22 (partial KLH2 domain d),

SEQ ID NO:23 (KLH2 domain g),
SEQ ID NO:24 (partial KLH2 domain h),
SEQ ID NO:49 (HtH1 domain a' + signal peptide),
SEQ ID NO:50 (partial HtH2 domain a),
SEQ ID NO:51 (HtH2 domain b'),
SEQ ID NO:52 (HtH2 domain d'),
SEQ ID NO:53 (HtH2 domain e'),
SEQ ID NO:54 (KLH1 domain e'),
SEQ ID NO:55 (KLH1 domain f),
SEQ ID NO:56 (KLH1 domain g),
SEQ ID NO:57 (KLH2 domain b'),
SEQ ID NO:58 (KLH2 domain c'),
SEQ ID NO:59 (KLH2 domain d'),
SEQ ID NO:60 (KLH2 domain e),
SEQ ID NO:61 (KLH2 domain f),
SEQ ID NO:62 (KLH2 domain g'),
SEQ ID NO:80 (HtH1 domain a'' + signal peptide),
SEQ ID NO:81 (HtH1 domain b''),
SEQ ID NO:82 (HtH1 domain c''),
SEQ ID NO:83 (HtH1 domain d''),
SEQ ID NO:84 (HtH1 domain e''),
SEQ ID NO:85 (HtH1 domain f''),
SEQ ID NO:86 (HtH1 domain g''),
SEQ ID NO:87 (HtH1 domain h''),
SEQ ID NO:88 (partial HtH2 domain a''),
SEQ ID NO:89 (HtH2 domain b''),
SEQ ID NO:90 (HtH2 domain c''),
SEQ ID NO:91 (HtH2 domain d''),
SEQ ID NO:92 (HtH2 domain e''),
SEQ ID NO:93 (HtH2 domain f''),
SEQ ID NO:94 (HtH2 domain g''),
SEQ ID NO:95 (HtH2 domain h''),
SEQ ID NO:96 (partial KLH1 domain b''),

SEQ ID NO:97 (KLH1 domain c"),
 SEQ ID NO:98 (KLH1 domain d"),
 SEQ ID NO:99 (KLH1 domain e"),
 SEQ ID NO:100 (KLH1 domain f"),
 SEQ ID NO:101 (KLH1 domain g"),
 SEQ ID NO:102 (KLH2 domain b"),
 SEQ ID NO:103 (KLH2 domain c"),
 SEQ ID NO:104 (KLH2 domain d"),
 SEQ ID NO:105 (KLH2 domain e"),
 SEQ ID NO:106 (KLH2 domain f"),
 SEQ ID NO:107 (KLH2 domain g"),
 SEQ ID NO:108 (partial KLH2 domain h"),
 SEQ ID NO:157 (complete Hth2 domain a);

- (b) nucleic acid sequences which hybridize with the counter-strand of a nucleic acid sequence according to (a) and code for a polypeptide which has the immunological properties of at least one domain of a haemocyanin;
- (c) nucleic acid sequences which on the basis of the genetic code are degenerated to the DNA sequences defined under (a) and (b) and code for a polypeptide which has the immunological properties of at least one domain of a haemocyanin;
- (d) nucleic acid sequences which hybridize with one of the nucleic acid sequences described under (a) to (c) and the counter-strand of which codes for a polypeptide which has the immunological properties of at least one domain of a haemocyanin;
- (e) nucleic acid sequences which are at least 60% homologous to one of the nucleic acid sequences described under (a);
- (f) variants of the sequences described under (a) to (d), the variants containing additions, deletions, insertions or inversions with respect to the sequences

described under (a) to (d) and coding for a polypeptide which has the immunological properties of at least one domain of haemocyanin; and

(g) combinations of several of the DNA sequences described under (a) to (f).

2. Nucleic acid molecule according to claim 1, **characterized in that** the intron sequence is selected from:

(i) nucleic acid sequences which are selected from the DNA sequences shown below or the corresponding RNA sequences or which contain these:

SEQ ID NO:109 (HtH1 intron 1S-1/1S-2),
 SEQ ID NO:110 (HtH1 intron 1S-2/1A-1),
 SEQ ID NO:111 (HtH1 intron 1A-1/1A-2),
 SEQ ID NO:112 (HtH1 intron 1A-2/1A-3),
 SEQ ID NO:113 (HtH1 intron 1A-3/1A-4),
 SEQ ID NO:114 (HtH1 intron 1A-4/1B),
 SEQ ID NO:115 (HtH1 intron 1B/1C),
 SEQ ID NO:116 (HtH1 intron 1C/1D),
 SEQ ID NO:117 (HtH1 intron 1D/1E),
 SEQ ID NO:118 (HtH1 intron 1E/1F-1),
 SEQ ID NO:119 (HtH1 intron 1F-1/1F-2),
 SEQ ID NO:120 (HtH1 intron 1F-2/1G-1),
 SEQ ID NO:121 (HtH1 intron 1F-1/1G-2),
 SEQ ID NO:122 (HtH1 intron 1G-2/1G-3),
 SEQ ID NO:123 (HtH1 intron 1G-3/1H),
 SEQ ID NO:124 (intron in the 3'UTR of HtH1),
 SEQ ID NO:125 (HtH2 intron 2A-1/2A-2),
 SEQ ID NO:126 (HtH2 intron 2A-1/2A-3),
 SEQ ID NO:127 (HtH2 intron 2A-1/2A-4),
 SEQ ID NO:128 (HtH2 intron 2A-4/2B),
 SEQ ID NO:129 (HtH2 intron 2B/2C),
 SEQ ID NO:130 (HtH2 intron 2C/2D),

SEQ ID NO:131 (HtH2 intron 2D/2E),
 SEQ ID NO:132 (HtH2 intron 2E/2F-1),
 SEQ ID NO:133 (HtH2 intron 2F-1/2F-2),
 SEQ ID NO:134 (HtH2 intron 2F-2/2GF-1),
 SEQ ID NO:135 (HtH2 intron 2G-1/2G-2),
 SEQ ID NO:136 (HtH2 intron 2G-2/2G-3),
 SEQ ID NO:137 (HtH2 intron 2G-3/2H),
 SEQ ID NO:138 (intron in the 3'UTR of HtH2),
 SEQ ID NO:139 (KLH1 intron 1B/1C),
 SEQ ID NO:140 (KLH1 intron 1C/1D),
 SEQ ID NO:141 (KLH1 intron 1D/1E),
 SEQ ID NO:142 (KLH1 intron 1E/1F),
 SEQ ID NO:143 (KLH1 intron 1F-1/1F-2),
 SEQ ID NO:144 (KLH1 intron 1F-2/1G-1),
 SEQ ID NO:145 (KLH1 intron 1G-1/1G-2),
 SEQ ID NO:146 (KLH1 intron 1G-2/1G-3),
 SEQ ID NO:147 (KLH2 intron 2B/2C),
 SEQ ID NO:148 (KLH2 intron 2C/2D),
 SEQ ID NO:149 (KLH2 intron 2D/2E),
 SEQ ID NO:150 (KLH2 intron 2E/2F),
 SEQ ID NO:151 (KLH2 intron 2F),
 SEQ ID NO:152 (KLH2 intron 2F-2/2G),
 SEQ ID NO:153 (KLH2 intron 2G-1/2G-2),
 SEQ ID NO:154 (KLH2 intron 2G-2/2G-3),
 SEQ ID NO:155 (KLH2 intron 2G/2H);

- (ii) nucleic acid sequences which hybridize with the counter-strand of a nucleic acid sequence according to (i);
- (iii) nucleic acid sequences which are at least 60% homologous to one of the nucleic acid sequences described under (i);

- (iv) variants of the sequences described under (i) to (iii), wherein the variants contain additions, deletions, insertions or inversions with respect to the sequences described under (i) to (iv); and
 - (v) combinations of several of the DNA sequences described under (i) to (iv).
3. Nucleic acid molecule according to claim 1 or 2, **characterized in that** the hybridization described under (b), (d) or (ii) is carried out under stringent conditions.
 4. Nucleic acid molecule according to claim 1 or 2, **characterized in that** the nucleic acid molecule described under (e) is at least 80% homologous to one of the nucleic acid sequences described under (a).
 5. Nucleic acid molecule according to claim 1 or 2, **characterized in that** the nucleic acid molecule described under (e) is at least 90 % homologous to one of the nucleic acid sequences described under (a).
 6. Nucleic acid molecule according to claim 1 or 2, **characterized in that** the nucleic acid molecule described under (e) is at least 95 % homologous to one of the nucleic acid sequences described under (a).
 7. Nucleic acid molecule according to claim 2, **characterized in that** the nucleic acid molecule described under (iii) is at least 80% homologous to one of the nucleic acid sequences described under (i).
 8. Nucleic acid molecule according to claim 2, **characterized in that** the nucleic acid molecule described under (iii) is at least 90% homologous to one of the nucleic acid sequences described under (i).
 9. Nucleic acid molecule according to claim 2, **characterized in that** the nucleic acid molecule described under (iii) is at least 95% homologous to one of the nucleic acid sequences described under (i).

10. Nucleic acid molecule according to one of claims 1 to 9, **characterized in that** it is a deoxyribonucleic acid molecule.
11. Construct comprising a nucleic acid molecule according to one of claims 1 to 10.
12. Construct according to claim 11, furthermore comprising a promoter which is suitable for expression control, the nucleic acid sequence which codes for a haemocyanin, a haemocyanin domain or a functional fragment thereof being under the control of the promoter.
13. Construct according to claim 11 or 12, furthermore comprising a nucleic acid sequence which codes for an antigen and is coupled directly to the nucleic acid sequence which codes for a haemocyanin, a haemocyanin domain or a functional fragment thereof.
14. Construct according to claim 13, wherein the antigen is selected from: tumour antigens, virus antigens and antigens of bacterial or parasitic pathogens.
15. Construct according to one of claims 11 to 14, wherein the construct comprises at least a part of a vector, the vector being selected from: bacteriophages, adenoviruses, vaccinia viruses, baculoviruses, SV40 virus and retroviruses.
16. Construct according to one of claims 11 to 15, wherein the construct furthermore comprises a His tag-coding nucleic acid sequence and the expression of the construct leads to the formation of a fusion protein with a His tag.
17. Host cell containing a construct according to one of claims 11 to 15, wherein the host cell is a prokaryotic or eukaryotic cell suitable for expression of the construct.
18. Host cell according to claim 17, **characterized in that** the prokaryotic host cell is selected from E. coli and Bacillus subtilis.

19. Host cell according to claim 17, **characterized in that** the eukaryotic host cell is selected from yeast cells, plant cells, insect cells and mammalian cells, preferably from CHO cells, COS cells and HeLa cells.
20. Process for the preparation of a haemocyanin polypeptide, wherein the nucleic acid molecule according to one of claims 1 to 10 and/or the construct according to one of claims 11 to 16 is expressed in a suitable host cell and the protein is isolated, if appropriate.
21. Process according to claim 20, **characterized in that** the haemocyanin polypeptide prepared is modified naturally or chemically.
22. Process according to claim 21, **characterized in that** the modification is a crosslinking or a covalent bonding to an antigen.
23. Process according to one of claims 20 to 22, **characterized in that** the expression is carried out in a host cell according to one of claims 17 to 19.
24. Haemocyanin polypeptide, comprising an amino acid sequence which is coded by one or more of the nucleic acid molecules according to one of claims 1 to 10.
25. Haemocyanin polypeptide according to claim 24, comprising at least one amino acid sequence selected from the following group:

SEQ ID NO:25 (HtH1 domain a + signal peptide),

SEQ ID NO:26 (HtH1 domain b),

SEQ ID NO:27 (HtH1 domain c),

SEQ ID NO:28 (HtH1 domain d),

SEQ ID NO:29 (HtH1 domain e),

SEQ ID NO:30 (HtH1 domain f),

SEQ ID NO:31 (HtH1 domain g),

SEQ ID NO:32 (HtH1 domain h),

SEQ ID NO:33 (partial HtH2 domain b),

SEQ ID NO:34 (HtH2 domain c),
SEQ ID NO:35 (HtH2 domain d),
SEQ ID NO:36 (HtH2 domain e),
SEQ ID NO:37 (HtH2 domain f),
SEQ ID NO:38 (HtH2 domain g),
SEQ ID NO:39 (HtH2 domain h),
SEQ ID NO:40 (partial KLH1 domain b),
SEQ ID NO:41 (KLH1 domain c),
SEQ ID NO:42 (partial KLH1 domain d),
SEQ ID NO:43 (partial KLH1 domain e),
SEQ ID NO:44 (KLH2 domain b),
SEQ ID NO:45 (KLH2 domain c),
SEQ ID NO:46 (partial KLH2 domain d),
SEQ ID NO:47 (KLH2 domain g),
SEQ ID NO:48 (partial KLH2 domain h),
SEQ ID NO:63 (HtH1 domain a' + signal peptide),
SEQ ID NO:64 (HtH1 domain h'),
SEQ ID NO:65 (partial HtH2 domain a),
SEQ ID NO:156 (complete HtH2 domain a),
SEQ ID NO:66 (HtH2 domain b'),
SEQ ID NO:67 (HtH2 domain d'),
SEQ ID NO:68 (HtH2 domain e'),
SEQ ID NO:69 (partial KLH1 domain b'),
SEQ ID NO:70 (KLH1 domain e'),
SEQ ID NO:71 (KLH1 domain f),
SEQ ID NO:72 (KLH1 domain g),
SEQ ID NO:73 (KLH1 domain h),
SEQ ID NO:74 (KLH2 domain b'),
SEQ ID NO:75 (KLH2 domain c'),
SEQ ID NO:76 (KLH2 domain d'),
SEQ ID NO:77 (KLH2 domain e),
SEQ ID NO:78 (KLH2 domain f),
SEQ ID NO:79 (KLH2 domain g'),

SEQ ID NO:158 (partial KLH2 domain h),

or a fragment of one of these sequences which has the immunological properties of at least one domain of a haemocyanin.

26. Recombinant haemocyanin polypeptide, obtainable by the process according to one of claims 20 to 23 or modifications thereof.
27. Recombinant haemocyanin polypeptide according to claim 22, **characterized in that** it comprises the sequences SEQ ID NO: 25 to 32 and is haemocyanin 1 from *Haliotis tuberculata*, it being possible for the sequence with SEQ ID NO:25 to be replaced by SEQ ID NO:63 and/or SEQ ID NO:32 to be replaced by SEQ ID NO:64.
28. Recombinant haemocyanin polypeptide according to claim 22, **characterized in that** it comprises either the sequences SEQ ID NO: 33 to 39 or the sequences SEQ ID NO:65, 66, 34-39 and is haemocyanin 2 from *Haliotis tuberculata*, it being possible in each case for SEQ ID NO:35 to be replaced by SEQ ID NO:67 and/or SEQ ID NO:36 to be replaced by SEQ ID NO:68.
29. Recombinant haemocyanin polypeptide according to claim 27, **characterized in that** it has an apparent molecular weight of 370 kDa in SDS-PAGE under reducing conditions.
30. Recombinant haemocyanin polypeptide according to claim 28, **characterized in that** it has an apparent molecular weight of 370 kDa in SDS-PAGE under reducing conditions.
31. Recombinant haemocyanin polypeptide according to claim 25, **characterized in that** the haemocyanin polypeptide comprises either the sequences SEQ ID NO: 40 to 43 or the sequences SEQ ID NO:40 to 43 and SEQ ID NO:71 to 73 and is KLH1 from *Megathura crenulata*, it being possible in each case for the sequence with SEQ ID NO:40 to be replaced by SEQ ID NO:66 and/or SEQ ID NO:43 to be replaced by SEQ ID NO:70.

32. Recombinant haemocyanin polypeptide according to claim 25, **characterized in that** the haemocyanin polypeptide comprises either the sequences SEQ ID NO: 44 to 48 or the sequences SEQ ID NO:44 to 46, 77, 78, 47, 48 and is KLH2 from *Megathura crenulata*, it being possible in each case for the sequence with SEQ ID NO:44 to be replaced by SEQ ID NO:74, SEQ ID NO:45 to be replaced by SEQ ID NO:75, SEQ ID NO:46 to be replaced by SEQ ID NO:76 and/or SEQ ID NO:47 to be replaced by SEQ ID NO:79.
33. Recombinant haemocyanin polypeptide according to one of claims 24 to 32, **characterized in that** it is bonded covalently to viruses, virus constituents, bacteria, bacteria constituents, DNA, DNA constituents, inorganic or organic molecules, such as e.g. carbohydrates, peptides and/or glycoproteins.
34. Recombinant haemocyanin polypeptide according to one of claims 24 to 33, **characterized in that** the haemocyanin polypeptide is non-glycosylated.
35. Recombinant haemocyanin polypeptide according to one of claims 24 to 33, **characterized in that** the haemocyanin polypeptide is glycosylated.
36. Pharmaceutical composition, comprising a nucleic acid molecule according to one of claims 1 to 10 and/or a construct according to one of claims 11 to 16 and physiologically tolerated additives.
37. Pharmaceutical composition according to claim 36, **characterized in that** it is used for gene therapy treatment of tumours.
38. Pharmaceutical composition, comprising a haemocyanin polypeptide according to one of claims 24 to 35 and physiologically tolerated additives.
39. Pharmaceutical composition according to claim 38, **characterized in that** it is used as an antiparasitic composition, antiviral composition or as an antitumour composition.

40. Pharmaceutical composition according to claim 38, **characterized in that** it is used for treatment of one of the following diseases: schistosomiasis, high blood pressure, surface bladder carcinomas, epithelial carcinomas, ovarian carcinoma, mammary carcinoma, bronchial carcinoma and colorectal carcinoma.
41. Pharmaceutical composition according to claim 38, **characterized in that** it is used as a vaccine.
42. Pharmaceutical composition according to claim 38, **characterized in that** it is used for prevention of cocaine abuse.
43. Use of a haemocyanin polypeptide according to one of claims 24 to 35 as a carrier substance for medicaments.
44. Liposome, comprising a nucleic acid molecule according to one of claims 1 to 10, a construct according to one of claims 11 to 16 and/or a haemocyanin polypeptide according to one of claims 24 to 35.
45. Liposome according to claim 44, **characterized in that** the liposome furthermore comprises cell recognition molecules.
46. Antibodies, obtainable by immunization of a test animal with the recombinant haemocyanin polypeptide according to one of claims 24 to 35.
47. Screening method for identification of tumour-specific DNA in a cell, comprising:
 - a) bringing cell DNA and/or cell protein into contact with a probe comprising the nucleic acid sequence according to one of claims 1 to 10 and/or the antibody according to claim 46 and
 - b) detecting the specific binding.

48. Screening method according to claim 47, **characterized in that** the tumour to be detected is a bladder carcinoma, epithelial carcinoma, ovarian carcinoma, mammary carcinoma, bronchial carcinoma or colorectal carcinoma.